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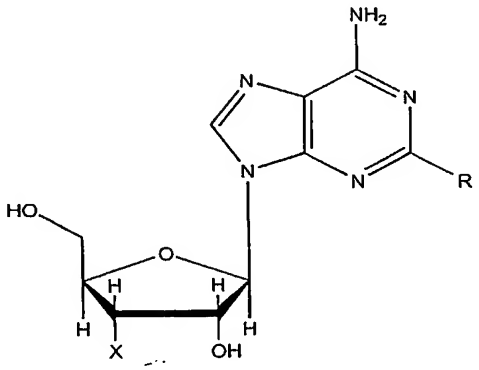

(I)

(57) Abstract: Use of compounds of formula: (I) wherein R is C_{1-4} alkoxy and X is H or OH; for the prevention, treatment, or amelioration of cancer, inflammation, auto-immune disease, ischemia-reperfusion injury, epilepsy, sepsis, septic shock, neurodegeneration (including Alzheimer's Disease), muscle fatigue or muscle cramp is described. The compounds are effective at very low doses, and so can be administered at doses at which serious side effects are not observed.

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USE OF ADENOSINE RECEPTOR AGONISTS IN THERAPY

This invention relates to use of adenosine receptor agonists as therapeutic compounds.

Adenosine is a ubiquitous local hormone/neurotransmitter that acts on four known receptors, the adenosine A1, A2A, A2B and A3 receptors. Adenosine generally serves to balance the supply and demand of energy in tissues. For example, in the heart released adenosine slows the heart by an A1 receptor mediated action in the nodes and atria (Belardinelli, L & Isenberg, G Am. J. Physiol. 224, H734-H737), while simultaneously dilating the coronary artery to increase energy (i.e. glucose, fat and oxygen) supply (Knabb et al., Circ. Res. (1983) 53, 33-41). Similarly, during inflammation adenosine serves to inhibit inflammatory activity, while in conditions of excessive nerve activity (e.g. epilepsy) adenosine inhibits nerve firing (Klitgaard et al., Eur J. Pharmacol. (1993) 242, 221-228). This system, or a variant on it, is present in all tissues.

Adenosine itself can be used to diagnose and treat supraventricular tachycardia. Adenosine A1 receptor agonists are known to act as powerful analgesics (Sawynok, J. Eur J Pharmacol. (1998) 347, 1-11). Adenosine A2A receptor agonists are known to act as anti-inflammatory agents (for example, from US 5,877,180 and WO 99/34804). In experimental animals, A2A receptor agonists have been shown to be effective against a wide variety of conditions including sepsis, arthritis, and ischaemia/reperfusion injury arising from renal, coronary or cerebral artery occlusion. The common factor in these conditions is a reduction in the inflammatory response caused by the inhibitory effect of this receptor on most, if not all, inflammatory cells.

However, the ubiquitous distribution of adenosine receptors means that administration of adenosine receptor agonists causes adverse side effects. This has generally precluded the development of adenosine-based therapies. Selective A1 receptor agonists cause bradycardia. The first selective A2A receptor agonist (2-[4-(2-carboxyethyl)phenylethylamino]-5'-N-ethylcarboxamidoadenosine, or CGS21680), was tested in a Phase 2A clinical trial as a potential anti-hypertensive. However,

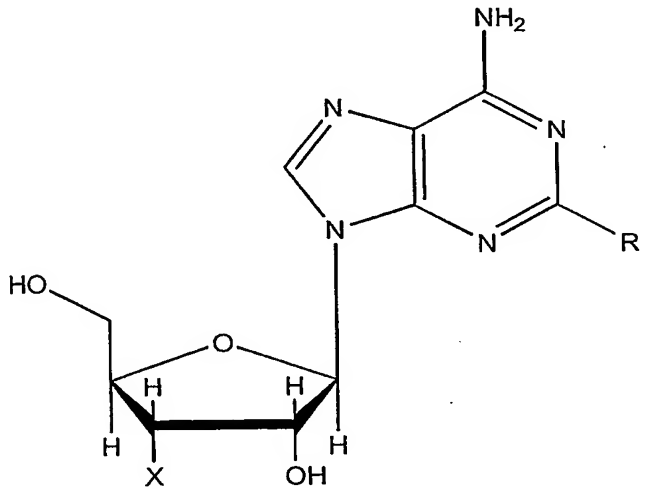
administration caused a large fall in blood pressure and consequent increase in cardiac output. FR 2162128 discloses that adenosine derivatives (including 2-alkoxy adenosine derivatives comprising a lower alkyl group of not less than two carbon atoms) have hypotensive and coronary vasodilatory activity.

Bartlett *et al* (J. Med. Chem. 1981, *24*, 947-954) discloses the evaluation of analogues of 1-methylisoguanosine. These analogues include 2-methoxyadenosine (also known as spongosine). This and other compounds were tested for their skeletal muscle-relaxant, hypothermic, cardiovascular and anti-inflammatory effects in rodents following oral administration (anti-inflammatory activity was assessed by inhibition of carageenan-induced oedema in a rat paw). 2-methoxyadenosine caused 25% inhibition of carageenan-induced inflammation in rats at 20 mg/kg po. However, reductions in mean blood pressure (41%), and in heart rate (25%) were also observed after administration of this compound at this dose.

There is, therefore, a need to provide adenosine receptor agonists that can be administered with minimal side effects.

According to the invention there is provided use of a compound of the following formula:

(I)

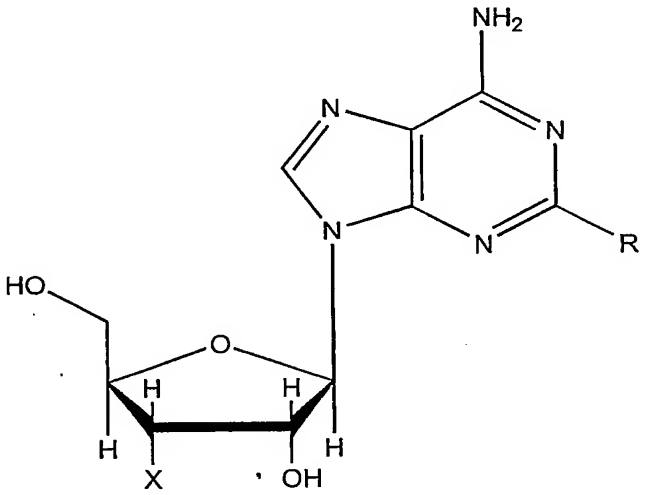


wherein R is C_{1-4} alkoxy and X is OH;

for the manufacture of a medicament for the prevention, treatment, or amelioration of cancer, inflammation, auto-immune disease, ischemia-reperfusion injury, epilepsy, sepsis, septic shock, neurodegeneration (including Alzheimer's Disease), muscle fatigue or muscle cramp (particularly athletes' cramp).

According to the invention there is also provided use of a compound of the following formula:

(II)



wherein R is C_{1-4} alkoxy, and X is H;
for the manufacture of a medicament for the prevention, treatment, or amelioration of cancer, inflammation, auto-immune disease, ischemia-reperfusion injury, epilepsy, sepsis, septic shock, neurodegeneration (including Alzheimer's Disease), muscle fatigue or muscle cramp (particularly athletes' cramp).

In particular, there is provided according to the invention use of a compound of formula I or II for the manufacture of a medicament for the prevention, treatment, or amelioration of inflammatory or auto-immune disease, including rheumatoid arthritis, osteoarthritis, rheumatoid spondylitis, gouty arthritis, and other arthritic conditions, psoriasis, asthma, chronic obstructive pulmonary disease, fibrosis, multiple sclerosis, endotoxic shock, gram negative shock, toxic shock, hemorrhagic shock, adult respiratory distress syndrome, cerebral malaria TNF-enhanced HIV replication,

TNF inhibition of AZT and DDI activity, organ transplant rejection, cachexia secondary to cancer, HIV, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcosis, bone resorption diseases, reperfusion injury (including damage caused to organs as a consequence of reperfusion following ischaemic episodes e.g. myocardial infarcts, strokes), autoimmune damage (including multiple sclerosis, Guillam Barre Syndrome, myasthenia gravis) graft v. host rejection, allograft rejections, fever and myalgia due to infection, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS related complex (ARC), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis and pyresis, irritable bowel syndrome, osteoporosis, cerebral malaria, bacterial meningitis, adverse effects from amphotericin B treatment, adverse effects from interleukin-2 treatment, adverse effects from OKT3 treatment, and adverse effects from GM-CSF treatment.

Compounds of formula (I) or (II) that are selective agonists of adenosine A2A and/or A3 receptors are particularly preferred because it is believed that such compounds will have strong anti-inflammatory activity. By selective agonists of adenosine A2A and/or A3 receptors is meant agonists that activate adenosine A2A and/or A3 receptors at concentrations that are lower (preferably one thousandth to one fifth) than required to activate adenosine A1 receptors. Furthermore, A1 receptors have pro-inflammatory activity, so such effects are expected to be minimised for compounds that are selective for A2A and/or A3 receptors.

Compounds of formula (I) include: 2-methoxyadenosine, 2-ethoxyadenosine, 2-propoxyadenosine, 2-isopropoxyadenosine, and 2-butoxyadenosine. Preferred compounds of formula (I) are 2-methoxyadenosine, 2-ethoxyadenosine, and 2-butyloxyadenosine.

Compounds of formula (II) include: 3'-deoxy-2-methoxyadenosine, 3'-deoxy-2-ethoxyadenosine, 3'-deoxy-2-propoxyadenosine, 3'-deoxy-2-isopropoxyadenosine, and 3'-deoxy-2-butoxyadenosine. Preferred compounds of formula (II) are 3'-deoxy-2-propoxyadenosine, 3'-deoxy-2-isopropoxyadenosine, and 3'-deoxy-2-butoxyadenosine.

2-methoxyadenosine has been reported to have an EC50 value at the adenosine A2A receptor of 3 μM (Daly, J.W. *et al.*, (1993) Pharmacol. *46*, 91-100). However, this compound surprisingly has profound anti-inflammatory activity at plasma concentrations of 0.2μM or less. At these low doses 2-methoxyadenosine has reduced probability and severity of side effects. 2-methoxyadenosine can be administered at concentrations at which it is effective as an anti-inflammatory, but which are below those at which side effects are observed.

Other compounds of formula (I) and compounds of formula (II) are also believed to be much more effective at low doses than other adenosine receptor agonists. Thus, it is expected that compounds of formula (I) and compounds of formula (II) can be effectively administered at doses at which they have reduced probability and severity of side effects, or at which side effects are not observed. Such compounds provide significant advantages over the vast majority of other adenosine receptor agonists which only have anti-inflammatory effects at the same concentrations at which serious side effects are observed.

Compounds of formula (I) or (II) may alternatively or additionally have reduced probability and severity of side effects compared to other adenosine receptor agonists.

The amount of a compound of formula (I) or (II) that is administered to a subject should be an amount which gives rise to a peak plasma concentration that is less than the EC50 value of the compound at adenosine receptors at pH 7.4.

It will be appreciated that the EC50 value of the compound is likely to be different for different adenosine receptors (i.e. the A1, A2A, A2B, A3 adenosine receptors). The amount of the compound that is to be administered should be calculated relative to the lowest EC50 value of the compound at the different receptors.

Preferably the peak plasma concentration is one thousandth to one fifth, or one fiftieth to one third (more preferably one thousandth to one twentieth, one hundredth or one fiftieth to one fifth, one fiftieth to one tenth, or one tenth to one fifth) of the EC50 value. Preferably the amount administered gives rise to a plasma concentration that is maintained for more than one hour between one thousandth and one fifth, more

preferably between one thousandth and one twentieth, or one hundredth and one fifth, or one fiftieth and one fifth, of the EC50 value of the compound at adenosine receptors at pH 7.4.

For the avoidance of doubt, the EC50 value of a compound is defined herein as the concentration of the compound that provokes a receptor response halfway between the baseline receptor response and the maximum receptor response (as determined, for example, using a dose-response curve).

The EC50 value should be determined under standard conditions (balanced salt solutions buffered to pH 7.4). For EC50 determinations using isolated membranes, cells and tissues this would be in buffered salt solution at pH 7.4 (e.g. cell culture medium), for example as in Daly *et al.*, Pharmacol. (1993) 46, 91-100), or preferably Tilburg *et al* (J. Med. Chem. (2002) *45*, 91-100). The EC50 could also be determined *in vivo* by measuring adenosine receptor mediated responses in a normal healthy animal, or even in a tissue perfused under normal conditions (i.e. oxygenated blood, or oxygenated isotonic media, also buffered at pH 7.4) in a normal healthy animal.

Alternatively, the amount of a compound of formula (I) or (II) that is administered may be an amount that results in a peak plasma concentration that is one thousandth to one twentieth, one thousandth to one third, more preferably one hundredth to one fifth, or one fiftieth to one tenth, of the Kd value at adenosine receptors.

It will be appreciated that the Kd value of the compound is likely to be different for different adenosine receptors (i.e. the A1, A2A, A2B, A3 adenosine receptors). The amount of the compound that is to be administered should be calculated relative to the lowest Kd value of the compound for the different receptors.

Preferably the amount of the compound that is administered is an amount that results in a plasma concentration that is maintained for at least one hour between one thousandth and one fifth, more preferably between one thousandth and one twentieth, or one hundredth and one fifth, or one fiftieth and one fifth, of the Kd value of the compound at adenosine receptors.

The Kd value of the compound at each receptor should be determined under standard conditions using plasma membranes as a source of the adenosine receptors

derived either from tissues or cells endogenously expressing these receptors or from cells transfected with DNA vectors encoding the adenosine receptor genes. Alternatively whole cell preparations using cells expressing adenosine receptors can be used. Labelled ligands (e.g. radiolabelled) selective for the different receptors should be used in buffered (pH7.4) salt solutions (see e.g. Tilburg et al, J. Med. Chem. (2002) 45, 420-429) to determine the binding affinity and thus the Kd of the compound at each receptor.

Alternatively, the amount of a compound of formula (I) or (II) that is administered may be an amount that is one thousandth to one fifth, or one fiftieth to one third (preferably one thousandth to one twentieth, or one hundredth or one fiftieth to one fifth) of the minimum dose of the compound that gives rise to bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the compound is to be administered. Preferably the amount is one tenth to one fifth of the minimum dose that gives rise to the side effects. Preferably the amount administered gives rise to a plasma concentration that is maintained for more than 1 hour between one thousandth and one twentieth, or one hundredth or one fiftieth and one fifth of the minimum dose that gives rise to the side effects.

Alternatively, the amount of a compound of formula (I) or (II) that is administered may be an amount that gives rise to plasma concentrations that are one thousandth to one fifth, or one fiftieth to one third (preferably one thousandth to one twentieth, or one hundredth or one fiftieth to one fifth) of the minimum plasma concentration of the compound that cause bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the compound is to be administered. Preferably the amount gives rise to plasma concentrations that are one tenth to one fifth of the minimum plasma concentration that causes the side effects. Preferably the amount administered gives rise to a plasma concentration that is maintained for more than 1 hour between one thousandth and one twentieth, or one hundredth or one fiftieth and one fifth, of the minimum plasma concentration that causes the side effects.

It is expected that the amount of a compound of formula (I) or (II) that is administered should be 0.01 to 15 mg/kg, for example 0.01 to 5 or 10 mg/kg. The

amount may be less than 6 mg/kg, for example 0.01 to 2 mg/kg. The amount may be at least 0.01 or 0.1 mg/kg, for example 0.1 to 2 mg/kg, or 0.2 to 1 mg/kg. A typical amount is 0.2 or 0.6 to 1.2 mg/kg.

Preferred doses for a 70kg human subject are less than 420mg, preferably at least 0.7mg, more preferably at least 3.5mg, most preferably at least 7mg. More preferably 7 to 70mg, or 14 to 70mg.

The dosage amounts specified above are significantly lower (up to approximately 100 times lower) than would be expected (based on the EC50 value of spongosine at the adenosine A2A receptor) to be required for the compounds of formula (I) to have any beneficial therapeutic effect.

The appropriate dosage of a compound of formula (I) or (II) will vary with the age, sex, weight, and condition of the subject being treated, the potency of the compound, and the route of administration, etc. The appropriate dosage can readily be determined by one skilled in the art.

Compounds of formula (I) and compounds of formula (II) may be particularly effective for the prevention, treatment, or amelioration of particular types of inflammation, including arthritis (particularly at the joint capsule of arthritis), asthma, psoriasis, and bowel inflammation.

Compounds of formula (I) and compounds of formula (II) may be particularly effective in the prevention, treatment, or amelioration of rheumatoid arthritis, irritable bowel syndrome or osteoarthritis.

There is further provided according to the invention a method of prevention, treatment, or amelioration of cancer, inflammation, ischemia-reperfusion injury, epilepsy, sepsis, septic shock, neurodegeneration (including Alzheimer's Disease), muscle fatigue or muscle cramp (particularly athletes' cramp), which comprises administering a compound of formula (I) or (II) to a subject in need of such prevention, treatment, or amelioration.

Embodiments of the invention relating to use of a compound of formula (I) (particularly for the prevention, treatment, or amelioration of inflammation) may exclude 2-methoxyadenosine.

Compounds of formula (I) or (II) may be administered with or without other therapeutic agents, for example analgesics (such as opiates, NSAIDs, cannabinoids, tachykinin modulators, or bradykinin modulators) or anti-hyperalgesics (such as gabapentin, pregabalin, cannabinoids, sodium or calcium channel modulators, anti-epileptics or anti-depressants).

In general, a compound of formula (I) or (II) may be administered by known means, in any suitable formulation, by any suitable route. A compound of the invention is preferably administered orally, parenterally, sublingually, transdermally, intrathecally, or transmucosally. Other suitable routes include intravenous, intramuscular, subcutaneous, inhaled, and topical. The amount of drug administered will typically be higher when administered orally than when administered, say, intravenously.

Suitable compositions, for example for oral administration, include solid unit dose forms, and those containing liquid, e.g. for injection, such as tablets, capsules, vials and ampoules, in which the active agent is formulated, by known means, with a physiologically acceptable excipient, diluent or carrier. Suitable diluents and carriers are known, and include, for example, lactose and talc, together with appropriate binding agents etc.

A unit dosage of a compound of the invention typically comprises 5 to 500 mg of the active agent. Preferably the active agent is in the form of a pharmaceutical composition comprising the active agent and a physiologically acceptable carrier, excipient, or diluent. The preferred dosage is 0.1 to 2, e.g. 0.5 to 1, typically about 0.2 or 0.6, mg of the active agent per kg of the (human) subject. At these levels, effective treatment can be achieved substantially without a concomitant fall (for example, no more than 10%) in blood pressure.

A preferred administration frequency of compounds of the invention is expected to be two or three times per day.

Compounds of the invention can also serve as a basis for identifying more effective drugs, or drugs that have further reduced side effects.

Embodiments of the invention relating to compounds of formula (I) may exclude 2-propoxyadenosine, and/or 2-isopropoxyadenosine.

Embodiments of the invention relating to compounds of formula (II) may exclude 3'-deoxy-2-methoxyadenosine and/or 3'-deoxy-2-ethoxyadenosine.

Embodiments of the invention are described in the following examples with reference to the accompanying drawings in which:

Figure 1 shows that 2-methoxyadenosine inhibits carrageenan induced inflammation without affecting blood pressure;

Figure 2 shows that 2-methoxyadenosine (0.6 mg/kg p.o.) has no significant effect on blood pressure or heart rate; and

Figure 3 shows the change in plasma concentration over time after administration of 2-methoxyadenosine.

Example 1

Figure 1: A. 2-methoxyadenosine (62.4 and 624 μg/kg i.p.) inhibits carrageenan (CGN) induced inflammation with comparable efficacy to indomethacin (3mg/kg, po), without affecting blood pressure. Carrageenan (2%, 10 microlitres) was administered into the right hind paw, and the paw volume assessed by plethysomometry. 2-methoxyadenosine was administered at the same time as carrageenan. 2-methoxyadenosine was as effective as indomethacin (Indo, 3mg/kg p.o.).

Example 2

Figure 2: An implantable radiotelemetry device was placed in the abdominal cavity of 6 rats per group. The pressure catheter of the device was inserted in the abdominal aorta and two electrodes tunnelised under the skin in a lead II position (left side of abdominal cavity/right shoulder). Individual rats were placed in their own cage on a radioreceptor (DSI) for data acquisition. The effect of 0.6mg/kg 2-methoxyadenosine or vehicle (p.o.) on blood pressure was then assessed. A: blood pressure; B: heart rate.

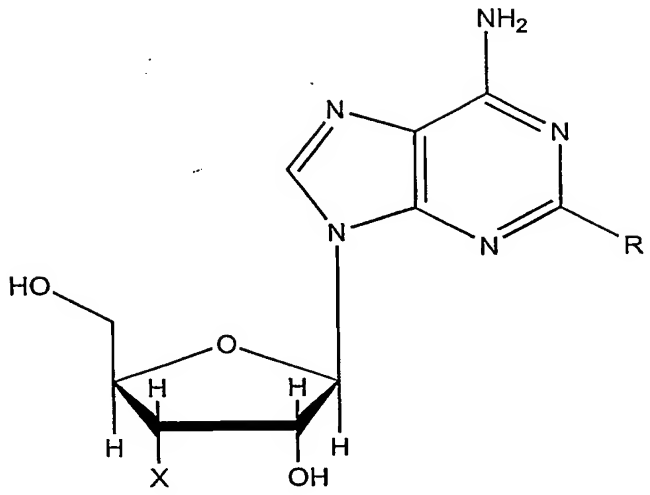
Example 3

The EC50 value of 2-methoxyadenosine at the adenosine A2A receptor is 900ng/ml (3 μM). Figure 3 shows the change in plasma concentration over time after administration of 2-methoxyadenosine at 0.6 mg/kg to a rat. It can be seen that the plasma concentration remains above 2% of the EC50 value for more than 3 hours. Anti-inflammatory effects have been observed (without blood pressure changes) when the peak and maintained plasma concentrations are as low 8ng/ml (i.e. 2% of the EC50 value determined in vitro). If the peak plasma concentration reaches the 900ng/ml level (i.e. the EC50 value) profound reductions in blood pressure occur that last for many hours.

Claims

1. Use of a compound of formula (I):

(I)

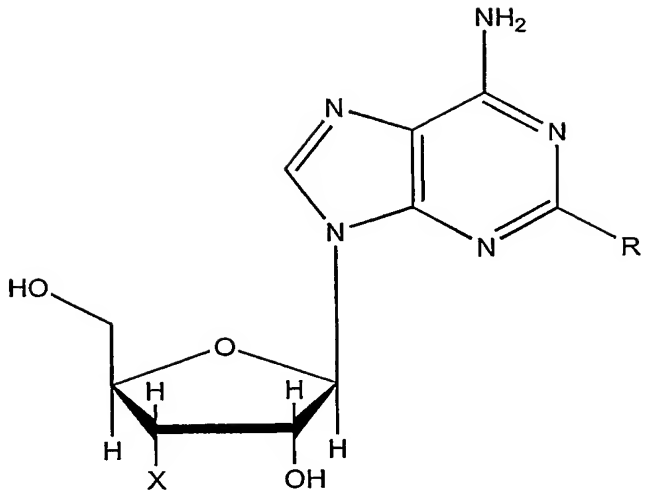


wherein R is C_{1-4} alkoxy and X is OH;

for the manufacture of a medicament for the prevention, treatment, or amelioration of cancer, inflammation, auto-immune disease, ischemia-reperfusion injury, epilepsy, sepsis, septic shock, neurodegeneration (including Alzheimer's Disease), muscle fatigue or muscle cramp at a dosage which, after administration to a subject, gives rise to a peak plasma concentration that is less than the EC50 value of the compound at adenosine receptors at pH 7.4

2. Use of a compound of formula (II):

(II)



wherein R is C_{1-4} alkoxy and X is H;
for the manufacture of a medicament for the prevention, treatment, or amelioration of cancer, inflammation, auto-immune disease, ischemia-reperfusion injury, epilepsy, sepsis, septic shock, neurodegeneration (including Alzheimer's Disease), muscle fatigue or muscle cramp at a dosage which, after administration to a subject, gives rise to a peak plasma concentration that is less than the EC50 value of the compound at adenosine receptors at pH 7.4.

3. Use according to claim 1, wherein the compound is 2-methoxyadenosine, 2-ethoxyadenosine, or 2-butyloxyadenosine.

4. Use according to claim 2, wherein the compound is 3'-deoxy-2-propoxyadenosine, 3'-deoxy-2-isopropoxyadenosine, or 3'-deoxy-2-butoxyadenosine.

5. Use according to any preceding claim for preventing, treating, or ameliorating arthritis, bowel inflammation, rheumatoid arthritis, osteoarthritis, rheumatoid spondylitis, gouty arthritis, and other arthritic conditions, psoriasis, asthma, chronic obstructive pulmonary disease, fibrosis, multiple sclerosis, endotoxic shock, gram

negative shock, toxic shock, hemorrhagic shock, adult respiratory distress syndrome, cerebral malaria TNF-enhanced HIV replication, TNF inhibition of AZT and DDI activity, organ transplant rejection, cachexia secondary to cancer, HIV, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcosis, bone resorption diseases, reperfusion injury (including damage caused to organs as a consequence of reperfusion following ischaemic episodes e.g. myocardial infarcts, strokes), autoimmune damage (including multiple sclerosis, Guillam Barre Syndrome, myasthenia gravis) graft v. host rejection, allograft rejections, fever and myalgia due to infection, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS related complex (ARC), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis and pyresis, irritable bowel syndrome, osteoporosis, cerebral malaria, bacterial meningitis, adverse effects from amphotericin B treatment, neurodegenerative diseases including Alzheimer's Disease, adverse effects from interleukin-2 treatment, adverse effects from OKT3 treatment, and adverse effects from GM-CSF treatment..

6. Use according to any preceding claim at a dosage that results in plasma concentrations being maintained for more than 1 hour between one thousandth and one fifth of the EC50 value of the compound at adenosine receptors at pH 7.4.

7. Use according to any of claims 1 to 5 at a dosage that results in plasma concentrations being maintained for more than 1 hour between one thousandth and one fifth of the minimum plasma concentration of the compound that gives rise to bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the compound is to be administered.

8. Use according to any preceding claim at a dosage of 0.01 to 15 mg/kg.

9. Use according to any preceding claim at a dosage of 0.1 to 2 mg/kg.

10. Use according to any preceding claim at a dosage of 0.6 to 1.2 mg/kg.

11. Use according to any preceding claim, wherein the medicament is in the form of a unit dose comprising 1 to 500 mg of the compound.

12. A pharmaceutical composition in unit dose form comprising up to 500mg of a compound as defined in any of claims 1 to 4, and a physiologically acceptable carrier, excipient, or diluent.

13. A method of prevention, treatment, or amelioration of cancer, inflammation, auto-immune disease, ischemia-reperfusion injury, epilepsy, sepsis, septic shock, neurodegeneration (including Alzheimer's Disease), muscle fatigue or muscle cramp, which comprises administering a compound as defined in any of claims 1 to 4 to a subject in need of such prevention, treatment, or amelioration at a dosage which gives rise to a peak plasma concentration in the subject that is less than the EC50 value of the compound at adenosine receptors at pH 7.4.

14. A method according to claim 13 for preventing, treating, or ameliorating arthritis, bowel inflammation, rheumatoid arthritis, osteoarthritis, rheumatoid spondylitis, gouty arthritis, and other arthritic conditions, psoriasis, asthma, chronic obstructive pulmonary disease, fibrosis, multiple sclerosis, endotoxic shock, gram negative shock, toxic shock, hemorrhagic shock, adult respiratory distress syndrome, cerebral malaria TNF-enhanced HIV replication, TNF inhibition of AZT and DDI activity, organ transplant rejection, cachexia secondary to cancer, HIV, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcosis, bone resorption diseases, reperfusion injury (including damage caused to organs as a consequence of reperfusion following ischaemic episodes e.g. myocardial infarcts, strokes), autoimmune damage (including multiple sclerosis, Guillam Barre Syndrome, myasthenia gravis) graft v. host rejection, allograft rejections, fever and myalgia due to infection, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS related complex (ARC), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis and pyresis,

irritable bowel syndrome, osteoporosis, cerebral malaria, bacterial meningitis, adverse effects from amphotericin B treatment, adverse effects from interleukin-2 treatment, adverse effects from OKT3 treatment, and adverse effects from GM-CSF treatment..

15. A method according to claim 13 or 14, wherein the compound is administered to the subject at a dosage that results in plasma concentrations of the compound in the subject being maintained for more than 1 hour between one thousandth and one fifth of the EC50 value of the compound at adenosine receptors at pH 7.4.

16. A method according to any of claims 13 to 15, wherein the compound is administered to the subject at a dosage that results in plasma concentrations of the compound in the subject being maintained for more than 1 hour between one thousandth and one fifth of the minimum plasma concentration of the compound that gives rise to bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the compound is to be administered.

17. A method according to any of claims 13 to 16, wherein the compound is administered at a dosage of 0.01 to 15 mg/kg.

18. A method according to any of claims 13 to 17, wherein the compound is administered at a dosage of 0.1 to 2 mg/kg.

19. A method according to any of claims 13 to 18, wherein the compound is administered at a dosage of 0.6 to 1.2 mg/kg.

20. Use of a compound as defined in any of claims 1 to 4 for discovery of drugs for the prevention, treatment, or amelioration of cancer, inflammation, ischemia-reperfusion injury, epilepsy, sepsis, septic shock, neurodegeneration (including Alzheimer's Disease), muscle fatigue or muscle cramp.

Figure 1

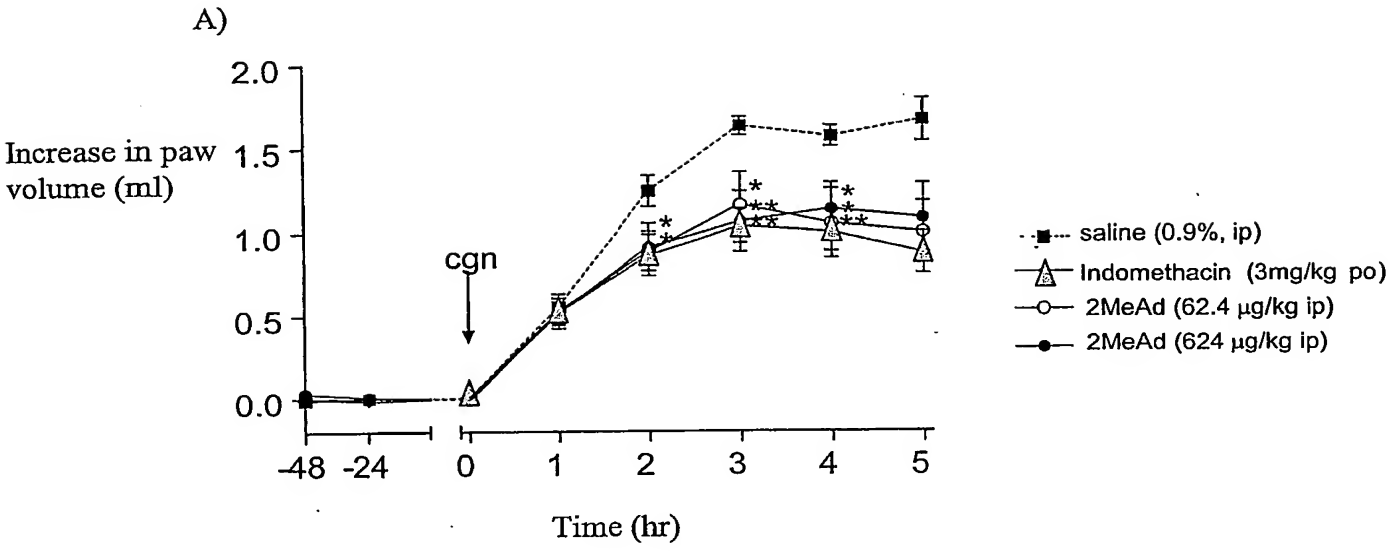
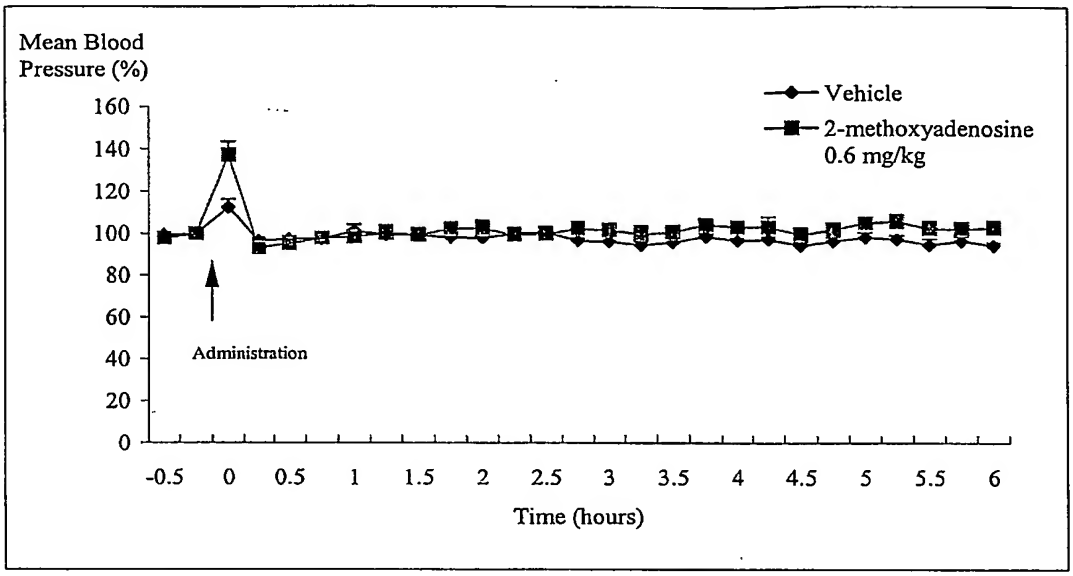

A)
Increase in paw volume (ml)
2.0
1.5
1.0
0.5
0.0
cgn
-48
-24
0
1
2
3
4
5
Time (hr)
saline (0.9%, ip)
Indomethacin (3mg/kg po)
2MeAd (62.4 µg/kg ip)
2MeAd (624 µg/kg ip)

Figure 2

A)


Mean Blood Pressure (%)
160
140
120
100
80
60
40
20
0
Vehicle
2-methoxyadenosine 0.6 mg/kg
Administration
-0.5 0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6
Time (hours)

B)

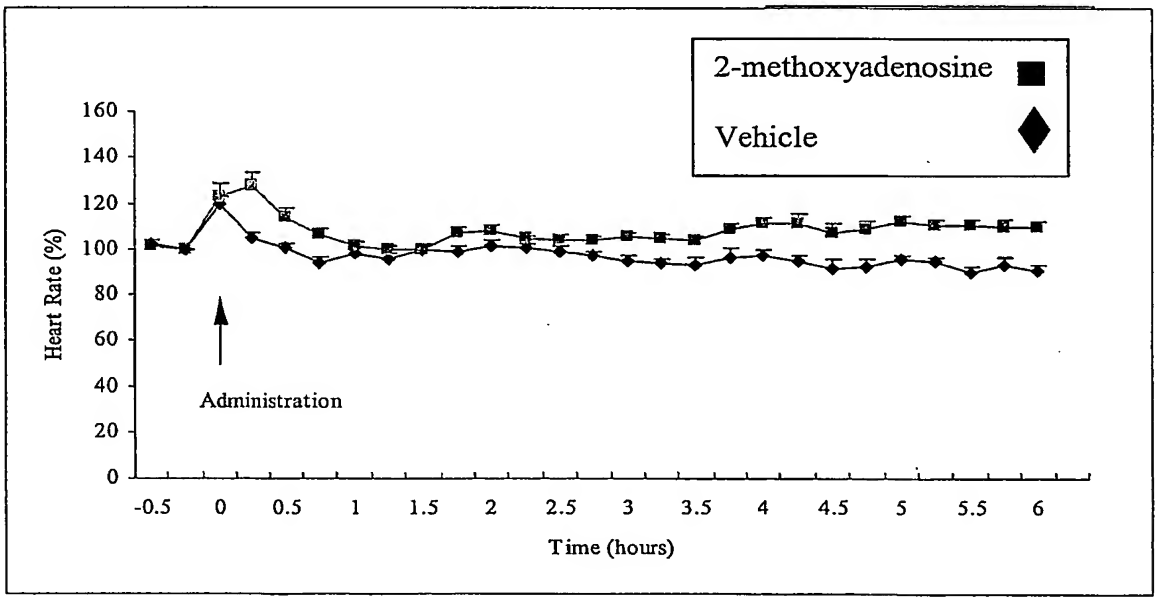
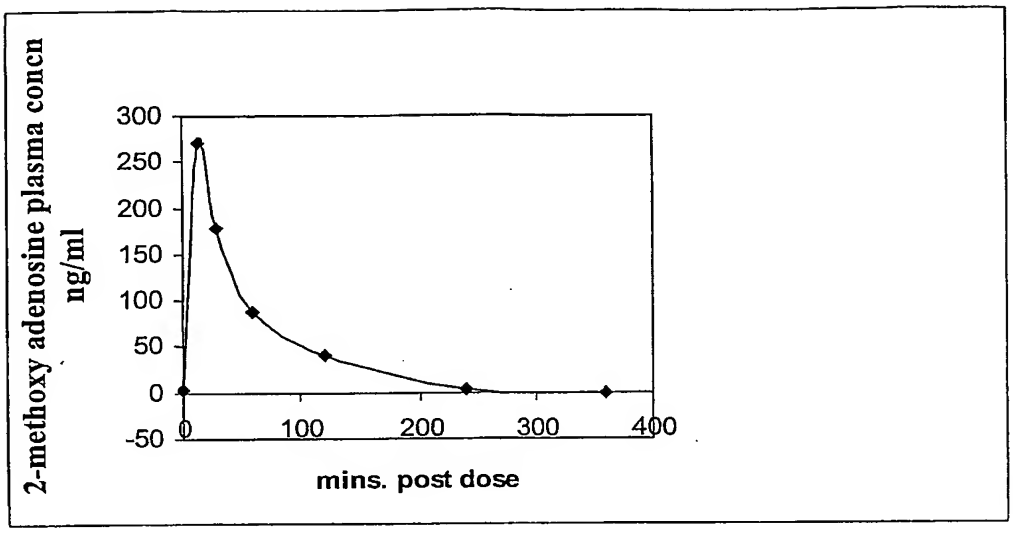

2-methoxyadenosine
Vehicle
Heart Rate (%)
160
140
120
100
80
60
40
20
0
Administration
-0.5 0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6
Time (hours)

Figure 3


2-methoxy adenosine plasma concn ng/ml
300
250
200
150
100
50
0
-50
0
100
200
300
400
mins. post dose

INTERNATIONAL SEARCH REPORT

Ir ıtional Application No
PCT/GB2004/000952

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/7076 A61P25/08 A61P25/28 A61P21/02 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/34804 A (UNIV VIRGINIA) 15 July 1999 (1999-07-15) cited in the application	1,5-19
Y	page 12, line 13 - line 30 claims 1-3 page 13, line 1 - line 7 page 14, line 5 - line 8	3
Y	RIEGER J M ET AL: "Design, Synthesis, and Evaluation of Novel A2A Adenosine Receptor AgonistS" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 44, 2001, pages 531-539, XP002222174 ISSN: 0022-2623 abstract page 531, left-hand column	3

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 July 2004	04/08/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Siatou, E

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 877 180 A (LINDEN JOEL M ET AL) 2 March 1999 (1999-03-02) cited in the application claims 1,2 column 5, line 47 - line 55	1,5-19
X	FR 2 162 128 A (TAKEDA CHEMICAL INDUSTRIES LTD) 13 July 1973 (1973-07-13) cited in the application claims 1-37 page 7, line 13 - line 18	12
A	UEEDA M ET AL: "2-Alkoxyadenosines: Potent and selective agonists at the coronary artery A2 adenosine receptor" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 34, 1991, pages 1334-1339, XP002225574 ISSN: 0022-2623 the whole document	1-19
A	BARTLETT R T ET AL: "Synthesis and pharmacological evaluation of a series of analogues of 1-methylisoguanosine" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 24, 1981, pages 947-954, XP002225573 ISSN: 0022-2623 cited in the application abstract page 947, right-hand column; figure I	1-19

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/GB2004/000952

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 13-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 20
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 13-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

Claims Nos.: 20

Claim 20 relates to the use of compounds of formula (I) or (II) in a method for discovering potential therapeutic agents. No further technical cahracteristics are given. Claim 20 therefore has not been the subject of a search

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/000952

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9934804 A	15-07-1999	AU 2108299 A	26-07-1999
		CA 2317093 A1	15-07-1999
		EP 1044004 A1	18-10-2000
		JP 2002500188 T	08-01-2002
		WO 9934804 A1	15-07-1999
		US 2002082240 A1	27-06-2002
		US 6514949 B1	04-02-2003
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		US 6514949 B1	04-02-2003
FR 2162128 A	13-07-1973	JP 48076894 A	16-10-1973
		JP 48061498 A	28-08-1973
		BE 792155 A1	30-05-1973
		DE 2258378 A1	14-06-1973
		ES 409140 A1	01-12-1975
		FR 2162128 A1	13-07-1973
		NL 7216299 A	05-06-1973
		AU 4941272 A	30-05-1974